ON MICRO-ORGANISMS IN TISSUES OF DISEASED HORSES.

By Dr. Oscar Katz.

Under date March 22nd last, Mr. E. Stanley, Veterinary Surgeon to the Government of New South Wales, reported on a disease which broke out among horses in the south-west of this colony, causing an alarming mortality among them. It would seem as if the disease was first noticed at Mingary, South Australia, early in December, 1886, but it is uncertain whether the horses attacked came from that colony or from New South Wales. It commenced to spread through railway contractors' teams, of which 40 animals out of 150 succumbed. There was at the time plenty of horse-labour employed, owing to the construction of a railway from Petersburgh (S.A.), to Silverton (N.S.W.), as well as to the extensive mining industries along the Barrier Ranges, and horse-owners not knowing anything about the character of the sickness and its treatment, being also anxious to escape the infected spots, caused the disorder to invade remote districts on the River Darling, and to go down to the south as far as Port Pirie. It is also said to have been carried by sea to Albany, Western Australia. At Silverton, a town in one of the silver-mining districts of the Barrier Ranges, it made its appearance on January 12th, 1887, and it was to this place that Mr. Stanley went to investigate it.

He describes it as an "epizootic contagious fever," the contagious matter of which, given off by the diseased through serous discharges from the body-orifices, principally those of the head, and through the faeces, is taken up by healthy individuals through contaminated food (water included). It does not affect other animals or man. Although resembling, to some extent, certain

horse-diseases known under the terms of "epizootic cellulitis," "rheumatic influenza," "pinkeye," "purpura haemorrhagica," "epizootic pneumonia," it differs from all of them considerably.

"The disease shows a disturbance of the vascular system, with alterations in the character of the blood which cause obstructions in the capillary vessels, followed by haemorrhagic spots, accompanied by organic complications, more or less severe."

The characteristic symptoms are: rapid pulse and breathing, high body-temperature, highly inflamed eyes, swollen head and limbs, rapid loss of flesh, associated with great debility.

Mild forms of the fever occur; convalescence after severe attacks progresses very slowly. The mortality was about 10 to 15 per cent. during the inquiry.

About the period of incubation the report says:—"From the time of exposure to infection, from three days to three weeks" (that means to say, as I understand it, from the moment of exposure, which may in a number of cases cover the moment of infection, till the first symptoms are discovered).

The pathological anatomy is as follows:—

"Hæmorrhagic spots and stellate patches of inflammation are diffused over both serous and mucous surfaces, effusions of serous lymph, and adhesive inflammation of the coverings of the lungs, heart, liver, and spleen; also serous effusions into cellular tissues of the limbs and head. In fatal cases, the inflammation is so intense as to obstruct the circulation; local mortification is speedily followed by death."

Post mortem examinations were made on four cases, with the following result:—

1. "Coach horse. Putrid lungs."

2. "Teamster's hack. Pleuritic inflammation and gelatinous effusion covering the pericardial sac; also slight enlargement and inflammation of the spleen."

3. "Teamster's mare, 5 years old. Ill three or four weeks. The spleen was very much enlarged and honey-combed, with purulent matter, and the lymphatic glands generally inflamed."

4. "Hack mare, 3 years old, foal at foot, ill about three weeks, with a spleen in the same condition."

"The small intestines in every case were healthy."

In two (Nos. 3 and 4) out of these cases Mr. Stanley preserved some pieces of spleen and some lymphatic (mesenteric) glands; besides he secured in capillary tubes, which were afterwards closed, samples of vein-blood, withdrawn from the living animal during the height of the fever. All these specimens were handed to me for examination from the Department of Stock, some time ago. I communicated my report to the Chief Inspector of Stock, but being of opinion that the subject under notice might be of some interest to members of this Society, and that a somewhat fuller account published in its Proceedings, might help in either identifying the disease as a possibly known one, or recognising it, if not so, in case it should make its appearance elsewhere, I wish to say what follows.

The fragments of spleen and the lymphatic glands were—so I was informed on inquiry—secured immediately after the death of the patients, and at once transferred to methylated spirits. About three months having elapsed when I obtained for examination these specimens, which were pretty well hardened, I did not think it necessary to try to cultivate any micro-organisms out of them; and I may as well state beforehand that the character of the micro-organisms found in sections, did not admit of any positive result. So I proceeded to prepare a series of sections, some time after having changed the methylated spirits for absolute alcohol.

I shall speak first of the result of the examination of the *mesenteric glands.*

Sufficiently and uniformly stained sections (for instance by Loeffler's alkaline methylene blue or by bismarck brown) exhibited

under high powers of the miscroscope, at first glance, two morphologically different forms of bacteria. Their relative number to one another was not the same in all the preparations made; in this section the one, in that section the other was predominant; in others again both were nearly equally distributed. Generally speaking, their numbers were enormous throughout, notably in the surrounding tissue or capsule of the organs in question, where they were packed in dense masses. In the interior of the gland they were found partly detached or in short lines, partly grouped in small colonies, or forming elongated, straight or curved tracts, an appearance which would make it probable that they were located in capillary vessels.

The first of these bacterial forms is very conspicuous by its size as well as by its behaviour when treated with aniline dyes. It is a bacillus, about ·003-·0045 mm. long, (that is on the average somewhat more than half the diameter of a human red blood-corpuscle), and about ·001 mm. wide. It has cylinder-shape, rounded off at the extremities; some few specimens show the central part or that part towards one of the ends very slightly thickened or swollen. On being stained and mounted *lege artis*, the bacilli offer a most peculiar appearance. There are two portions or divisions easily distinguishable in them. The one, of from a third to a half of the length of the entire rods, stands out very prominently by being deeply stained; it occupies the one end of the latter, and it is only seldom that this portion is situated some little distance away from the end part of that half of the rods. The other portion or division proves to be stained only at its periphery, and only very faintly. In this way the organisms appear as capitate rods, yet the width of the chromatophilous heads does not exceed that of the rods in general. One might also say, these microbes appear, in the coloured preparations, under the image of a sheath which contains that intensely coloured portion at one end. This

portion cannot be a spore, because it can be stained by the ordinary aniline dyes within a short time, and without further trouble.

Noteworthy is that these bacilli retain the colour on being treated after Gram's method (solution of aniline water and gentian-violet; solution of iodine in iodide of potassium; absol. alcohol). On being stained with aniline water—gentian-violet, or—fuchsin, and then transferred to a solution of hydrochloric acid (as used in staining tubercle-bacilli), they give off the colour again. Double or contrast stains may easily be obtained. Tolerably fair preparations were obtained by a dilute solution of gentian-violet, and by after-staining with picro-lithion-carmine. Far better results, however, were derived from transferring the sections first to a solution of picro-lithion-carmine for $\frac{1}{2}$-$\frac{3}{4}$ of an hour, at about 30°C., next, after having been washed a short while in dilute alcohol, to aniline-gentian-violet (s. above), for half-an-hour at the same temperature; hereafter rinsing a little with alcohol, then allowing Gram's solution of iodine to act for about one minute and a-half; absol. alcohol; oil of cloves; Canada balsam.* The micro-organisms then appear dark blue on a pinkish underground. Equally satisfactory and very instructive preparations are obtainable by first colouring the section with aniline-gentian-violet for about $\frac{3}{4}$ of an hour at about 30°C.; washing a moment in alcohol, then using the iodine-solution for one minute and a-half; absol. alcohol until colour is no longer given off; dilute watery solution of eosine for 1-2 minutes; mixture of absol. alcohol and oil of cloves; oil of cloves; Canada balsam.† After this process the organisms come out deeply blue, while the tissue-elements (and another form of bacteria, s. below), assume a handsome pink colour.

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*Cf. Biondi, Die pathogenen Micro-organismen des Speichels. Zeitschr. f. Hygiene. Band II., Heft 2, Leipzig, 1887, p. 201.

†Cf. Biondi, l.c.

Finally, after having stained the sections after Gram (see above), I have tried successfully a contrast-stain by means of dilute solutions of vesuvin or bismarck-brown, in which the sections were kept about one minute. Afterwards I found the bacilli under consideration again of an intense blue, the tissue yellowish brown. Among the bacilli there were, here and there, specimens in which that portion showing but a faint colour reaction, and losing this little of colour by Gram's method, presents now a distinct though faint brownish or yellowish tint, in contra-distinction to the other portion with its intense blue colour.

The second form of bacteria are also bacilli of the same length, but as a rule, of only about one-half to two-thirds of the width of the former. As regards their outlines and their relation to the tissue, they behave in much the same way as those, with which they are either mixed or not. But their protoplasmic contents do not exhibit that peculiar differentiation into two portions as seen there; here and there, it is true, specimens occurred which presented a granular or fragmentary protoplasmic interior.

Without attempting to utter a definite opinion as to whether this bacterial form No. II. is a kind by itself, or merely represents a certain stage in the development of the other, No. I., I surmise that the latter is the case, seeing that the staining reaction of Bacillus II. resembles that of part of Bacillus I., and finding also, on close examination, apparently transition-forms between the two. In sections which were stained after Gram's process, and afterwards by brown colours (see above), I noticed that a great many bacilli, which otherwise resembled No. I., differed from them by having the chromatophilous portion less distinct, and now taken possession of by a brownish colour.

The question whether these bacteria occurring in the mesenteric glands, must be regarded as *the* cause or one of the causes of the horse-disease at issue, or whether they had made their appearance in those organs after the appearance of the disease, but during the life

of the respective individuals, cannot be definitely settled by what I was able to ascertain. However, it is not at all impossible, and I rather incline to that view, that as in typhoid fever, the occurrence of these micro-organisms in the mesenteric glands may be interpreted. I do not think it probable for them to be merely accidental. I want especially to draw attention to the peculiar morphological features of the bacteria, which I do not remember to have ever seen in preparations or figures, or noticed in descriptions.

Sections out of the fragments of *spleen*, which offered on the cut-surface a marbled or "honey-combed" appearance, caused by greyish-dirty necrotised masses alternating with brownish-red tissue (as seen in alcohol), yielded no such bacteria as did the mesenteric glands, but more or less numerous aggregations of another kind. It consists of streptococci. They readily stain with aniline dyes, for instance Loeffler's alkaline methylene-blue. On employing Gram's method (s. above), one finds them to remain coloured, and it is in this way that one procures the finest and most instructive preparations. In a section thus prepared one sees, at a low amplification (for instance of 70 diam.), a number of deep-blue foci amid the yellowish-grey tissue of the spleen, and irregularly distributed in the same. In some preparations they were very plentiful, in others scarce. They are of an irregular, roundish or elongated shape, in the latter case up to ·3 mm. long, whereas the smallest groups measure ·01 mm. and still less. Under high powers these groups or foci are found to be made up of aggregations of minute, about ·00045 mm. large, isodiametrical cocci (hence they are about the fourteenth part of the diameter of a human red blood-corpuscle). As a rule, they form more or less elongated strings or chains, which are interlaced with one another in different ways. Such chains are especially distinct at the margins of the aggregations; in the interior of the latter, particularly if dense, the micrococci are often isolated or in two's. Besides these masses which, as such, can

be rendered visible by low magnifications, one observes in going over the sections with an immersion-lens, detached chains in large numbers. They look very delicate, are bent differently, and embrace in some cases up to 30 links. These do not touch each other immediately, but are separated from each other by bright interspaces of about half the diameter of the cocci.

Their occurrence in the spleen extends not only to the necrotic parts, but also, though apparently less numerous, to the tissue which still contains well colourable nuclei; in sections stained with alkaline methylene-blue there were some groups of the streptococci undoubtedly disintegrated or about to disintegrate. We are, I think, pretty well justified in assuming that the presence of these necrotic masses in the spleen is due to the action of the described micrococci. We have analogies enough of this kind. But whether or not these micro-organisms are identical with one of the kinds of streptococci already known as infectious to man and animals (*e. g. Streptococcus pyogenes*) is impossible to decide after the mere morphological appearances of the concerning micro-organisms. Although the size of the streptococci under treatment is larger than that of the known kinds of infectious streptococci, yet this criterion cannot be regarded as absolutely decisive.

Finally a few words about the sample of blood alluded to in the beginning.

This blood had been withdrawn from a living individual while in the acute stage of the fever, into capillary tubes, which were afterwards hermetically closed. When I went to examine it for micro-organisms, it had been in the tubes for about four months. To the naked eye it appeared as a homogeneous liquid.

One portion of it I stained, and examined it under the microscope with the result that a moderate number of micrococci were found, which were arranged in small heaps without forming chains

These organisms, being besides a little larger than the streptococci in the spleen, are therefore morphologically different from the latter.

Another portion of the blood was used for cultivation purposes. On being transferred on an inclined surface of nutrient gelatine in test-tubes, it gave rise to a pure culture of micrococci similar to those in the blood. The cultures grew but slowly, being at the beginning greyish, then orange, and ultimately assuming a bright coral-red colour. The cultures did not liquefy the gelatine. They resembled to some extent, *Micrococcus cinnabareus* (Flügge, Micro-organismen, Leipzig, 1886, p. 174), and had, so to say, not the look of being infectious. Still I inoculated with such gelatine-cultures of the first, second, and third generations, six house-mice subcutaneously, of which four died, one of them after somewhat less than twenty-four hours, one within 30-44 hours, the third after forty-five hours, and the fourth after ten days. I doubt whether the inoculated culture had anything to do with the death of this latter animal. With some heart-blood of the first-mentioned mouse, which died in less than twenty-four hours, another mouse was infected; it died after about twenty-four hours. In this way I continued to inoculate from mouse to mouse in two other cases; death each time ensued after about the same time (twenty-four hours). Want of mice caused me to interrupt those experiments. There were no characteristic or constant pathological changes noticeable in the organs of the dead animals. A microscopic examination of, and cultivation experiments with, blood and sap of organs yielded negative results. The inoculated micrococci were never found there; however from the place of inoculation these micro-organisms were obtained. According to this result, no infection had taken place in the mice experimented upon, and the fatal results with most of them must be considered due to some toxic substance or substances elaborated by the multiplying organisms. These, then, are not infectious, at least not for mice; no doubt they were

derived from germs which, as contamination, found their way into the capillary tubes, somehow or other, when the sample of blood was collected. Here they grew for some time till the supply of oxygen present was exhausted. It is remarkable that they revived, after four months' imprisonment in the hermetically sealed tubes, on being transferred on to fresh nutrient material. I may mention, without any further going into details of the behaviour of this kind of micrococcus, that, when some of the original blood containing it, was uniformly distributed in liquefied gelatine (1·5 p.c. grape sugar in it), which was then solidified, colonies made their appearance only at the gelatine-surface, and a little below it; but here they remained insignificant. Thus this pigment-producing microbe furnishes another example of exclusively aërobic bacteria.